A mathematical model of HiF-1α-mediated response to hypoxia on the G1/S transition

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A mathematical model of HIF-1α-mediated response to hypoxia on the G1/S transition

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Abstract

Hypoxia is known to influence the cell cycle by increasing the G1 phase duration or by inducing a quiescent state (arrest of cell proliferation). This entry into quiescence is a mean for the cell to escape from hypoxia-induced apoptosis. It is suggested that some cancer cells have gain the advantage over normal cells to easily enter into quiescence when environmental conditions, such as oxygen pressure, are unfavorable [43, 1]. This ability contributes in the appearance of highly resistant and aggressive tumor phenotypes [2].

The HiF-1α factor is the key actor of the intracellular hypoxia pathway. As tumor cells undergo chronic hypoxic conditions, HiF-1α is present in higher level in cancer than in normal cells. Besides, it was shown that genetic mutations promoting overstabilization of HiF-1α are a feature of various types of cancers [8]. Finally, it is suggested that the intracellular level of HiF-1α can be related to the aggressiveness of the tumors [53, 24, 4, 10].

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However, up to now, mathematical models describing the G1/S transition under hypoxia, did not take into account the HiF-1α factor in the hypoxia pathway.

Therefore, we propose a mathematical model of the G1/S transition under hypoxia, which explicitly integrates the HiF-1α pathway. The model reproduces the slowing down of G1 phase under moderate hypoxia, and the entry into quiescence of proliferating cells under severe hypoxia. We show how the inhibition of cyclin D by HiF-1α can induce quiescence; this result provides a theoretical explanation to the experimental observations of Wen et al. (2010). Thus, our model confirms that hypoxia-induced chemoresistance can be linked, for a part, to the negative regulation of cyclin D by HiF-1α.

Keywords: Hypoxia, HiF-1α, G1/S transition, quiescence, cell cycle arrest, cancer

1. Introduction

The integration of environmental factors influencing cell proliferation is the actual challenge of cell cycle modeling [12]. Indeed, since the 1990's, many models were developed to describe the evolution of protein levels during the cycle [36, 11, 5, 52]. However, few of them take into account external parameters, such as the temperature, the mechanical properties of the substrate, or hypoxia. This last factor is particularly interesting to study. In physiological conditions, hypoxia can occur if the blood does not bring enough oxygen to the cells. If hypoxia is too severe or too long, the cell enters into apoptosis [23]. In pathological conditions, cancer cells undergo a chronic hypoxia [30, 31]. This hypoxia induces more aggressive, metastatic and resistant

tumors [23, 25]. In particular, if hypoxia can induce apoptosis in normal proliferating cells, tumor cells resist to apoptosis [23]. One mechanism that can explain this resistance is the entry into a quiescent state, where the cell stops its division cycle [43, 2]. This quiescent state is also a mean for the cancer cell to escape from the effects of chemotherapy [2, 33, 40]. Therefore, it participates to the aggressiveness of the tumors.

Alarcon *et al.* [1] proposed a simple model explaining why cancer cells can enter into quiescence under hypoxic conditions whereas normal cells follow their cycle. The authors introduce a difference between normal proliferating and cancer cells through the deregulation of the expression of a protein of the cell-cycle (p27) during tumorigenesis. However, this deregulation event is not a general feature of cancer. Indeed, this phenomenon was not observed in the majority of tumor cells [32, 39]. Besides, it is not clear that p27 is necessary to induce hypoxia-induced cell cycle arrest [6, 21].

The HiF-1α factor is the central protein involved in the intracellular signaling pathway of hypoxia [46]. It is a transcription factor, which enhances the expression of numerous genes. These genes enable the cell to adapt to the environmental conditions (angiogenesis, arrest of aerobic metabolism), or to enter into apoptosis [20, 37].

HiF-1 is an heterodimeric protein, constituted of two sub-units: HiF-1β, which is constitutively expressed, and HiF-1α, which is the sensor of hypoxia. Indeed, in order to activate hypoxia genes, HiF-1α has to be in a reduced form. When the level of oxygen is sufficient, an enzyme called HiF-1α prolyl-hydroxylase is active and converts HiF-1α into an hydroxylated form. This oxygenated form of HiF-1α is rapidly degraded by the proteasom pathway

[23, 44, 40].

The ways of actions of HiF-1α are numerous and complex [38]. A review of the huge molecular biology literature dealing with the effects of HiF-1α on the cell cycle enables us to retain several types of actions. First, it is clear that HiF-1α indirectly downregulates cyclin E activity, and this inhibition is the reason why HiF-1α causes slowing down or arrest of cell cycle [22, 19, 1]. The origins of this action on cyclin E activity remain poorly defined [22], even if some potential pathways are known. Notably, the upregulation of cyclins inhibitors, such as p21 and p27, are reported [17, 22, 20]. However, some authors showed that the action of HiF-1α on p27 is not so clear since the expression of p27 under hypoxia may be independent of HiF-1α [6, 9].

The second important effect of HiF-1α is the interrelation between this factor and cyclin D [50, 16]. Wen et al. [50] studied the effect of HiF stabilization on cyclin D level. They first found that after 24h at 0.2%, the mean cylin D concentration in the whole cell population decreases with 50% compared to the normoxic condition. To confirm the implication of HiF-1, its activity was impaired by DN-HiF overexpression, which induces an increase of the cyclin D level. There is also an action of cyclin D on HiF-1α, due to the activation of the HiF-1 prolyl-hydroxylase activity [16]. Besides, the activation of cyclin D expression by HiF-2 underlines the rivalry in hypoxic tumor growth and progression between HiF-1 and HiF-2. Here, we choose to focus on the influence of cyclin D inhibition by HiF-1α, in order to show how this simple relationship can generate a serie of interesting results in agreement with data from the literature. Besides, the inhibition of the cyclins by HiF-1α under hypoxia is very well documented. As we previously said,

hypoxia-dependent inhibition of cyclin E is considered as a cause of hypoxia-dependent cell-cycle arrest. Second, the increase of unphosphorylated versus phosphorylated form of the Retinoblastoma protein [19, 20] under hypoxia is also a good evidence for the downregulation of cyclin D by HiF.

The increase of HiF-1α activity during tumorigenesis is well documented, since it seems to be a very common feature of cancers [53, 29, 7, 42]. As a consequence, this factor became a new therapeutic target [45, 47]. In some cases, its high level is simply due to the chronic hypoxia undergone by the tumor cells. In other cases, genetic mutations induce an over-stabilization of HiF-1. It is the case for the renal clear carcinoma [13]. As a consequence, for the same oxygen pressure, HiF-1 will have a higher level in cancer cells than in normal cells [15].

In a general way, a link was found between the aggressiveness of cancers and HiF-1α activity. This role of HiF in tumorigenesis is firstly due to the induction of anti-apoptotic and pro-angiogenic genes. Second, it mediates the entrance into quiescence of proliferating cells, which induces a hypoxia-dependent chemoresistance.

The aim of this work is to describe an example of a simple mechanism of HiF-1α-dependent entrance into quiescence under hypoxia. We make a link between the level of HiF-1α and the ability of the cell to enter into quiescence under hypoxia. In agreement with the literature, our model assumes the regulation of HiF-1α stability by the oxygen pressure [51, 44, 34]. We built a mathematical model of the G1/S transition in hypoxic conditions, which explicitly integrates the HiF-1α pathway. We focused on the relationships between HiF-1α and the cyclins, in order to propose a simple and biologically

accurate mechanism of hypoxia-induced quiescence.

2. Model

2.1. Hypotheses

Our model is primary based on the models described in Alarcon *et al.* [1], Tyson and Novak [49], Novak and Tyson [36]. In those papers, the G1/S transition is modeled by a biological switch between a cyclin and an inhibitor complex. They considered that this inhibitor was APC/cdh1. However, this role of APC/cdh1 complex is not well admitted; classicaly, the complex addressing cyclin E for degradation is known to be the SCF complex [14]. As a consequence, it is more accurate to consider that the inhibitor of cyclin E is SCF. The switch that we aim to study is represented in Figure 1. The G1 phase finishes when this molecular switch occurs. In our model, we differentiate the cyclin E and the cyclin D. The cyclin E is involved in the molecular switch where it inhibits and is inhibited by the SCF complex. Whereas Novak and Tyson [36] consider that two cyclins drive the G1/S transition (cyclin E and A), we consider here just one cyclin we named cyclin E. The concentration in active SCF complex is controlled by an evolution equation similar to that given by Tyson and Novak [49] for the APC/cdh1 complex. The cyclin D phosphorylates the Rb protein, which releases the transcription factor E2F. As Novak and Tyson [36], we supposed that at each time, $E2F_{Rb}$ is in an equilibrium relationship with E2F and Rb because the complexation/decomplexation of phopshorylated Rb and E2F are supposed to be very fast compared to the evolution of the cyclin concentration. Besides, free E2F can be in an unphosphorylated (active) form, or in a phosphorylated

(inactive) form. A dynamic equilibrium ensures the transition from phosphorylated to unphosphorylated forms. The total concentration of E2F (free phosphorylated/unphosphorylated, and complexed with Rb) is supposed to be constant, as in [36]. Active E2F factor promotes the synthesis of the cyclin E, as assumed for the cyclin A by Novak and Tyson [36]. Besides, to take into account the cell growth in the progression through the G1 phase, we assume that the mass increases the cyclin E concentration. This assumption is taken from Alarcon et al. [1] and Novak and Tyson [36]. The oxygen pressure is taken into account with the variable P, which represents the percentage of oxygen among all the other gases in the cell environment. The effect of the oxygen pressure is modeled by the concentration of HiF-1α. This concentration increases when the oxygen pressure decreases, as well described in the literature [27, 54]. Jiang et al. [27] show that *in vitro* cultured cells submitted to hypoxia exhibit an exponential relationship between the HiF-1 level and the oxygen pressure [27]. The experimental results from Zhou et al. [54] confirm that an exponential law is a good model for the hypoxia-induced HiF-1 stabilization. These authors also showed that this exponential law strongly depends on the cancer cell lines. We will discuss this point later. HiF-1α decreases the cyclin D activity by inhibiting its synthesis, as observed in experiments [50]. The evolution of the cyclin D level during the cycle, the influence of HiF-1α on its level, and the reaction of Rb phosphorylation are calibrated with data from the literature. We do not take into account the role of the p27 protein, contrary to Alarcon *et al.* [1]. As we said, the effects of hypoxia on this protein remain unclear; besides, we want to focus on the influence of cyclin D/HiF interactions which promote the entrance into

quiescence. Figure 2 gives a sketch of the molecular network.

2.2. Biological description

The variables used in the model are the cyclin D ($cycD$), the cyclin E ($cycE$), the Retinoblastoma protein (in its unphosphorylated form) (Rb), HiF-1α (H), the SCF complex (SCF), the mass of the cell, and the different chemical forms of the E2F transcription factor. E2F exists in phosphorylated and unphosphorylated ($E2F$) form, and free or linked to Rb ($E2F_{Rb}$). Thus, free E2F can be phosphorylated or unphosphorylated. The free and unphosphorylated form ($E2F_A$) is the active form. A chemical equilibrium drives the transition between the phosphorylated and unphosphorylated forms of E2F. We named $E2F_{tot}$ the total of all the chemical forms of E2F. As Novak and Tyson [36], we assume this total to be constant. The cyclin D liberates E2F by phosphorylating Rb (that is to say by decreasing $[Rb]$). $E2F_A$ has a crucial role, since it promotes the synthesis of the cyclin E. Cell growth is the second factor influencing the cyclin E synthesis, trough the variable m. HiF-1 inhibits the cyclin D synthesis. As a consequence, hypoxia induces a decrease of $E2F_A$ maximal level, which decreases the synthesis rate of the cyclin E. The core of the model is the molecular switch between the cyclin E and its inhibitor (SCF). Following the same idea as Novak and Tyson [36] and Alarcon et al. [1], this switch is driven by the mutual inhibition of SCF and the cyclin E.

2.3. Mathematical formulation

The model can be written with a system of coupled ordinary differential equations (ODEs).

The cyclin D is synthesized with rate a_1 and naturally degraded with rate a_2. We assume that it is linearly decreasing with $[H]$ [50]:

$$\frac{d[cycD]}{dt} = a_1 - a_3[H] - a_2[cycD] \quad (1)$$

The synthesis of cyclin E is induced by the cell growth and $E2F_A$, according to Novak and Tyson [36] and Alarcon et al. [1]. Its degradation is stimulated by the SCF complex:

$$\frac{d[cycE]}{dt} = b_1 m[E2F_A] - b_2[cycE] - b_3[cycE][SCF] \quad (2)$$

The SCF complex obeys the same equation as the APC/cdh1 complex of the previous models [1, 36, 49]:

$$\frac{d[SCF]}{dt} = \frac{e_1(1-[SCF])}{J_1+1-[SCF]} - e_2\frac{[SCF][cycE]}{J_2+[SCF]} \quad (3)$$

As in Alarcon et al. [1], Rb (unphosphorylated Rb) is synthetized with a d_2 rate, and naturally degraded with a d_3 rate. The cyclin D phosphorylates Rb. As a consequence, $[Rb]$ decreases:

$$\frac{d[Rb]}{dt} = d_2 - (d_3 + d_1[cycD])[Rb] \quad (4)$$

The growth of the cell mass follows the same law as in Alarcon et al. [1], with a growth rate α and a maximal size m_0:

$$\frac{dm}{dt} = \alpha m(1 - \frac{m}{m_0}) \quad (5)$$

The unphosphorylated form of E2F follows a dynamic equilibrium described by Novak and Tyson [36]:

$$\frac{d[E2F]}{dt} = g_1([E2F]_{tot} - [E2F]) \quad (6)$$

The equilibrium relationship between the oxygen pressure and the HiF-1α concentration has been derived from experiments [27]:

$$[H] = H_0 e^{\beta_1(1-P)} \tag{7}$$

Following Novak and Tyson [36], we assume that at each time, the active E2F is the fraction of unphosphorylated ($\frac{[E2F]}{[E2F]_{tot}}$) free ($[E2F]_{tot} - [E2F_{Rb}$) E2F factor:

$$[E2F_A] = \frac{([E2F]_{tot} - [E2F_{Rb}])[E2F]}{[E2F]_{tot}} \tag{8}$$

The equilibrium between $E2F_{Rb}$, $E2F$ and Rb is given by the equation from Novak and Tyson [36]:

$$[E2F_{Rb}] = \frac{2[E2F]_{tot}[Rb]}{[E2F]_{tot} + [Rb] + \sqrt{([E2F]_{tot} + [Rb])^2 - 4[E2F]_{tot}[Rb]}} \tag{9}$$

As in previous models, the core of the system is the molecular switch between a cyclin and SCF. Mathematically, we consider that the cell passes through the G1 phase when $[cycE] > [cycE]_{threshold}$ and $[SCF] < [SCF]_{threshold}$. We chose $[SCF]_{thresold} = 0.004$ and $[cycE]_{thresold} = 0.15$. When these values are reached, the cell divides.

Inhibition of the cyclin D by HiF-1α. Eq.(1) and (7) describe the relationship between the cyclin D, HiF-1α and the oxygen pressure P in the cell environment. This model considers the effects of a long term (almost 48h) hypoxia. Thus, the level of HiF is stabilized. The cyclin D is synthesized with rate a_1, and degraded with rate a_2. Its synthesis is inhibited by HiF-1α, which concentration depends on oxygen pressure following the expression given in eq. (7). The parameter a_3 represents the action of HiF on the synthesis of cyclin

D. The parameter β_1 is determined from Jiang *et al.* [27] (see Appendices A). The variable P stands for the oxygen pressure in the cell environment, expressed as a normalized percentage of all the gases. As suggested by Jiang *et al.* [27], we consider that the reference pressure (P=1) is 6% O_2. All the pressures will be normalized with this reference pressure. The parameters a_1, a_2 and a_3 are estimated with data coming from Wen *et al.* [50] and Yang *et al.* [52] (see Appendix A).

Control of E2F concentration. We denote $[E2F]_{tot}$ the total concentration of E2F, $E2F_{RB}$ the complex E2F/Rb, $E2F$ the unphosphorylated form of E2F and $E2F_A$ the free and unphosphorylated (active) form. We describe the equilibrium between these different variables from Eqs.(6),(8),(9) previously proposed by Novak and Tyson [36]. The first describes the transition, mediated by g_1, from phosphorylated to unphosphorylated E2F. The others are equilibrium equations modeling the relationship between the different chemical species involving E2F.

Eq.(4) describes the evolution of the unphosphorylated Retinoblastoma protein concentration. $[Rb]$ is synthesized with coefficient d_2, naturally phosphorylated in the cell environment with coefficient d_3, and phosphorylated by the cyclin D with coefficient d_1.

Molecular switch. Eqs.(2),(3) describe the biological switch which forms the core of the model for the G1/S transition. Besides, we suppose that the synthesis of cyclins E, represented by the variable $cycE$ in our model, depends on the mass m and on the transcription factor E2F in its active form ($E2F_A$) [36]. The parameter b_1 regulates the speed of the chemical synthesis. In the

other hand, *cycE* is naturally degraded with coefficient b_2, and suffers SCF-mediated degradation with coefficient b_3. The aim is to simulate a switch similar to that obtained in these previous models of the G1/S transition. As in Novak and Tyson [36], the dynamics of the inhibitor depends on the cyclin E, and on the transition between an active and an inactive form. These dependencies are expressed through Michaelis-Menten terms with enzymatic constants J_1 and J_2 respectively. The parameter e_1 promotes the transition from inactive to active forms, and e_2 the transition from active to inactive forms.

Parameters. The parameters we used are given in Table 1. Their values were estimated from previous models [1, 36] and experimental data of molecular biology taken from the literature (see Appendix A). We will show that we can reduce the variability of all the parameters to the variability of e_2 and α, which control the dynamics of the system. These parameters can be easily adjusted to biological data.

3. Results

We simulated the G1/S transition by solving the system of equations Eqs.(1)-(9) for different values of P. We used a standard fourth order Runge-Kutta method, implemented in Matlab. The values of the parameters used in our first simulations are given in Table 1. We chose the undetermined parameters in order to have a G1 phase duration of about 400 minutes. This duration was chosen based on the work of Novak and Tyson (2004) [36]. The rise of cyclins D and A levels, which was interpreted by Novak and Tyson, and by ourselves, as the signal for the G1/S transition, occurs about 6-7

hours after the beginning of the cycle. The initial conditions we used are given in Table 2. The initial values for the concentrations were chosen to have values of the same order of magnitude to that used by Alarcon et al. [1] and Novak [36]. We can notice that the initial concentrations do not influence the final state of the system (see Appendices B). We consider that the cell passes through the G1 phase when $[cycE] > [cycE]_{threshold} = 0.15$ and $[SCF] < [SCF]_{threshold} = 0.04$. These threshold conditions were chosen to capture as precisely as possible the moment of the switch between the cyclin E and the SCF complex. In the rest of the paper, the notation $[X]_\infty$ stands for the value of $[X]$ when the system reaches its steady state.

3.1. Hypoxia induces entrance into quiescence

Figure 3 shows simulations of the G1/S transition under three levels of hypoxia. When P diminishes, the molecular switch occurs with a delay which increases when hypoxia becomes more severe. For a certain value of P (here, 0.01), this switch does not occur any more. As in [1], this inability for the cell to pass through the G1 phase can be interpreted as the entry into the quiescent state. P^* denotes the minimal pressure which enables the cell to pass through the G1 phase.The parameters are given in Table 1, and $P^* = 0.03$. We also plotted the duration of the G1 phase as a function of P (Figure 4). As hypoxia becomes more intense, the G1 phase duration increases, in agreement with the literature [17]. For the lowest value of the pressure, we observe an asymptotic behavior, which finishes by the entrance into quiescence.

In order to mathematically characterize the final state of the cell (quiescent or proliferating), the stationary state solutions of the system, depending

on P, are numerically calculated. The details of the calculation are presented in the Appendices B. Figure 5 presents the stable stationary states of the system as a function of P. When $P < P^*$, the system exhibits three stable stationary states. With our initial conditions ($[SCF]$ high and $[cycE]$ low), the equilibrium state of the system is reached with $[SCF] > [SCF]_{threshold}$ and $[cycE] < [cycE]_{threshold}$. This situation corresponds to the quiescent state. When $P > P^*$, the system has a unique stationary state. It is characterized by a high level of $cycE$ and a low concentration of SCF, which leads the cell to achieve its cycle.

3.2. Influence of parameters variability

Previous observations enable us to suppose that the two main features of the cell response to hypoxia are the increased duration of the G1 phase and the capacity to enter into quiescence. The latter can be quantified by the value of P^* (pressure for the bifurcation of the dynamics system). The core of the system is the molecular switch due to SCF and $cycE$ interactions. The variations of P have an influence on these dynamics by changing $[E2F_A]$ and m. Since these two variables determine the synthesis rate of $cycE$, they affect the G1 phase duration and equilibrium values of SCF and $cycE$.

The equilibrium value of m never changes in our model. We call this maximal mass value m_0, and we have m_∞=m_0. We can simulate its influence on the dynamics of the system by modifying b_1, which buffers the variations of m_0 in the production rate of $cycE$. Therefore, we do not consider m_0 as a key parameter for the dynamics of the system. The variations of the growth rate, by modifications of the parameter α, just have an influence on the cell cycle duration.

Final concentrations of the SCF and $cycE$ directly depend on $[E2F_A]_\infty$. We can express $[E2F_A]_\infty$ as a function of $[cycD]_\infty$, d_1, d_2 (see Appendix B). $[cycD]_\infty$ depends on the parameters of Eq.(1) and (7) (a_1, a_2, a_3, β_1). As a consequence, the variability of all these parameters is totally buffered by the variability of b_1, which controls the production rate of $cycE$.

As it ensures the coupling between SCF and $cycE$, the parameter e_2 also controls the dynamics of the molecular switch. If the coupling is weak, it is more difficult for the system to generate a molecular switch. As a consequence, we can also consider the parameter e_2 as a key factor influencing the behavior of the system.

Finally, the whole variability of the adjustment parameters can be simulated by the variability of the parameters e_2, b_1 and α.

Figure 6 shows that the (b_1, e_2) couple determines the value of P^*. When e_2 decreases, the coupling between SCF and $cycE$ becomes weak, and it is necessary to increase the hypoxic level to observe quiescence. As a consequence, P^* decreases. In parallel, when b_1 decreases, the production rate of $cycE$ at the steady state is less important. Therefore, the cell enters more easily into quiescence, and P^* increases. We note that, for a given value of b_1, the variations of e_2 are sufficient to simulate a large panel of values for P^*. As a consequence, in order to adjust the model to a given value of this limit pressure, we can set b_1 and consider e_2 as an adjustment parameter. Finally, the parameters controlling the dynamics of the system are e_2 (coupling between cyclin E and SCF) and α (growth rate).

4. Discussion

This paper proposes a mathematical model of the G1/S transition in hypoxic conditions. Our model was calibrated with data from the literature, and the results from other mathematical models of the G1 phase transition [1, 36]. The core of this model is a molecular switch between a cyclin and an inhibitor (SCF). The passage through the G1 phase is determined by the possibility to realize this switch. If the switch does not occur, we consider that the cell is trapped into a quiescent state. Hypoxia was simulated by introducing HiF-1α. We modeled the inhibition between cyclin D and HiF-1α, in agreement with data from the literature [50]. The oxygen pressure determines, in this way, the final concentration of cyclins D during the G1 phase. This level of cyclins D determines the maximum value of the active E2F concentration, which controls the synthesis rate of cyclins E. If this rate is not high enough, the critical concentration for the cyclin E is not reached and the molecular switch cannot occur. The cell enters into a quiescent state. As a consequence, if the oxygen pressure is low, the cyclin D will not reach a sufficient level to ensure the minimal amount of cyclins E the cell needs to achieve the G1 phase. We could define a limit pressure P^*, which is the minimum oxygen pressure whereby the cell can pass through the G1 phase. Mathematically, it corresponds to the bifurcation point of our system.

Thus, this model illustrates a simple mechanism of hypoxia-mediated slowing down of the cell cycle, and entrance into quiescence. It focuses on the influence of the interactions between HiF-1α and the cyclins on the G1 phase achievement. Notably, we show how the inhibition of cyclin D by HiF-1α can affect the cell cycle. By inhibiting the cyclin D activity, HiF promotes the

entrance into quiescence. Thus, it provides a resistance to apoptosis for the proliferative cells. This result gives an explanation to the results of Wen *et al.* [50], which have shown that the HiF-mediated chemoresistance appearing in tumor cells is linked to the inhibition of the cyclin D. Indeed, they observed that the inhibition of cyclin D expression by siRNA induces an increase of the resistance to chemically-induced apoptosis. They concluded than the negative regulation of the cyclin D induced by HiF can, at least partially, explain the chemoresistance which is associated to cancerous HiF-1 over-stabilization. Thus, our model shows how a certain form of chemoresistance can be associated to HiF-1 in cancer cells. In the case of a similar response to hypoxia between normal and cancer cells (non-mutated hypoxia pathway), the model highlights a possible mechanism for the chronic hypoxia-induced entry into quiescence.

In the case of a genetic over-stabilization of HiF-1, which is the case for various type of cancers [13, 8], the increased ability of cancer cells to enter into a quiescent state is well described by our model. Indeed, this case can be modeled by multiplying $[H_0]$ by an $\epsilon > 1$ parameter to artificially increase the HiF-1 level. As a consequence, for a same oxygen pressure, HiF-1 concentration will be more important in cancer cells than in normal cell. Thus, for a given hypoxic condition, a cancer cell can enter into a quiescent (resistant) state whereas the normal cells continue their cycle (more sensitive to apoptosis). Our model thus captures the differential behavior of cancer and normal cell. We can notice than this ϵ parameter could depend on the cell line, since Zhou et al. [54] showed that different cancer cell lines does not have the same normoxic level of HiF-1.

Compared to the model from Alarcon et al. [1], our work describes in a very general way the influence of HiF-1 on hypoxia-induced quiescence of normal and cancer cells. Whereas the previous model was based on an arguable action of hypoxia on p27, we constructed a biologically more accurate system of ODEs. This new model gives a possible explanation to the link between HiF-1, the cyclins and hypoxia-induced chemoresistance. Besides, our model can easily be completed to study the influence of a genetic overstabilization of HiF-1 by considering an ϵ parameter, which can vary with the considered cell line.

The α parameter, which drives the growth speed of the cell, was chosen to obtain a value of 400min for the G1 phase duration. It is important to notice that the results of our model are not influenced by the choice of the normoxic G1 phase duration. Since it does not influence the final state of the mathematical system, this α parameter can be modified to adjust the model to various G1 phase durations. It is biologically correct, because the duration of the G1 phase in the absence of an environmental stress is known to be, at least for a part, driven by the growth rate of the cell [5].

Besides, the influence of HiF-1 on the cell metabolism was not taken into account in this model. It is known that the glycolitic switch described by Gatenby and Gillies (2004) [18] is linked to the activity of HiF-1. Notably, it was shown that HiF-1 stimulates the synthesis of glycolitic enzymes, such as Pyruvate Kinase [37]. In parallel, Icreverzi et al. [26] observed that the cyclin D enhances the cell growth through the stimulation of mitobiogenesis. By inhibiting the synthesis of cyclins D, HiF-1 makes the mitobiogenesis to decrease, which promotes the transition to a glycolytic phenotype. This switch

allows cancer cells to compensate low aerobic metabolism by an increased glucose consumption. This can be a way for the cell to escape the quiescence state. It could be interesting to add to our model a term describing the energetic production, and to link the cell growth to this available energy.

We also have to notice that our model only studies the effect of a given oxygen pressure on a single cell. In the reality of tumor growth, there is a gradient of oxygen which varies in space and time. To study the effects of such a dynamical hypoxic condition, it would be necessary to consider some series of cycles, by resetting the values of the variables to their initial values after the G1/S transition is reached. For each new cycle, the oxygen level can change, and the cell response may also change.

Besides, it is possible to extend this model to consider a population of interacting cells. These interactions can be, for instance, through the diffusion of growth factors which stimulate cell proliferation by enhancing the synthesis of cyclins D [51]. We can also consider the differential mechanical interactions between the tumor cells, and between tumor cells and the stroma. These mechanical interactions are known to influence cell division [41].

We can notice here that the kinetics of HiF-1 accumulation is not considered in this model. We study the idealized case of a constant hypoxia, and we consider the effect of the equilibrium value of HiF-1. However, these kinetics were measured ([35, 28]). HiF-1 shows a peak after 6h, and then decreases to stabilize at an equilibrium level after 24-48h. This equilibrium level depends on the intensity of hypoxia, and on the cellular type (normal and cancer cells). If we consider the more complex case of a dynamical hy-

poxia, it should be necessary to take into account these kinetics of HiF-1 accumulation.

We note that the exact relationships between HiF-1 and the cyclin D is not totally elucidated. Many reports show that HiF-1 inhibits the cyclin D [50, 16] and the cyclin E [22, 19, 1] leading to the cell cycle arrest. However, an other report [3] shows that HiF-1 can also stimulate the induction of cyclins D, leading to cell proliferation. In our work, we use the first biological hypothesis, and we show that it can explain the cell cycle arrest phenomenon that is commonly observed.

Finally, we aim to calibrate the undetermined parameters with experimental data, by measuring the G1 phase duration under hypoxia and the value of P^*. Since we showed that only two parameters (α and e_2) drive the dynamics of the system (G1 phase duration and entrance into a quiescence state), experimental data will enable us to set the values of these parameters for different cell lines. Therefore, this would provide a tool to compare different cancer cell lines on the basis of their ability to enter into a quiescence state. It could be interesting to build up a classification of different cancer cells using the values of the parameters of our model. By comparing it with a known classification made on the basis of their resistance and aggressiveness, we could obtain some information about the correlation between the ability to enter into quiescence under hypoxia and the aggressiveness.

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We are very grateful to Françoise Giroud for her advices which helped us to build and justify the hypotheses of the model.

parameter	Value	Reference
a_1	0.51	[48], [36]
a_2	1	[48], [36]
$a_3 H_0$	0.0085	[50]
β_1	2.5	[27]
b_1	0.018	
b_2	0.5	[1]
b_3	1	[1]
J_1	0.04	[1]
J_2	0.04	[1]
d_1	0.2	[36]
d_2	0.1	[1]
e_2	14	
e_1	1	
α	0.005	
g_1	0.016	[36]
$[\mathrm{E2F}]_{tot}$	1	
$[cycE]_{threshold}$	0.15	[1]
$[SCF]_{threshold}$	0.004	[1]

Table 1: Parameters used in the model. The parameters we could not estimate are initially determined in order to reach a G1 phase duration of about 400 *min* for proliferative cells in normoxic conditions

Model's variables	Notations	Initial Value
Masse	m	5
Cyclin D	$cycD$	0.1
Non-phosphorylated Rb	Rb	1
Cyclin E	$cycE$	0.01
SCF complex	SCF	0.9
Unphosphorylated E2F	$E2F$	0.1

Table 2: Initial values taken for the simulations

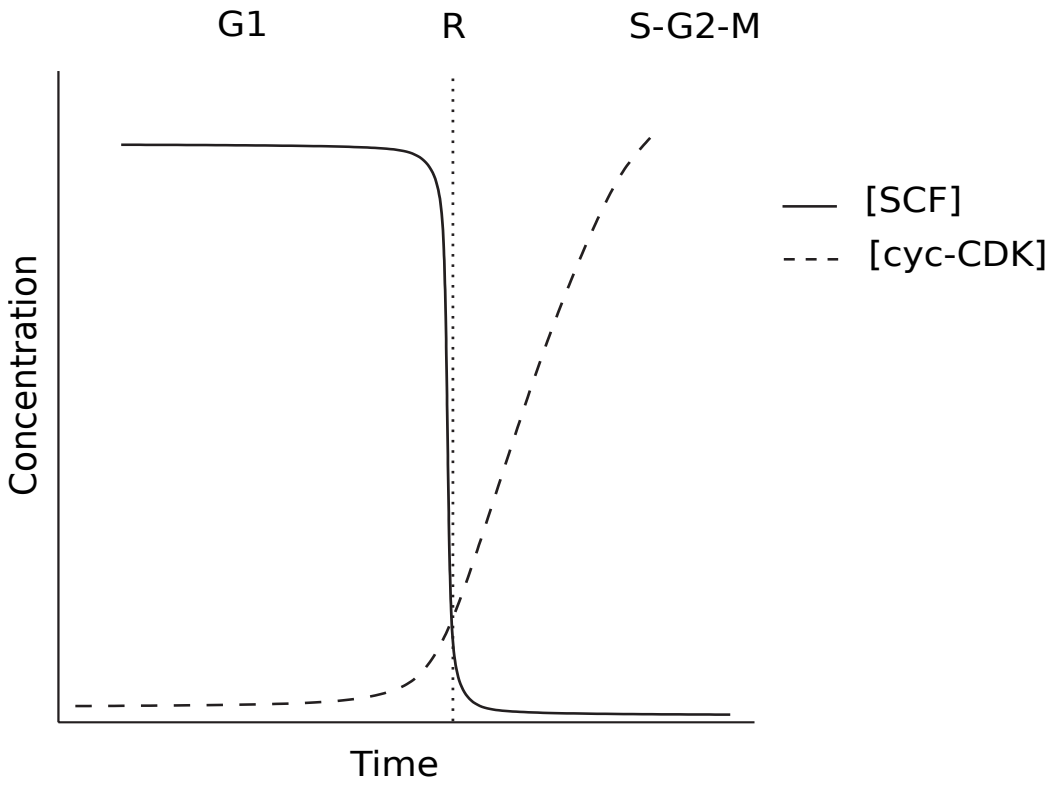

Figure 1:

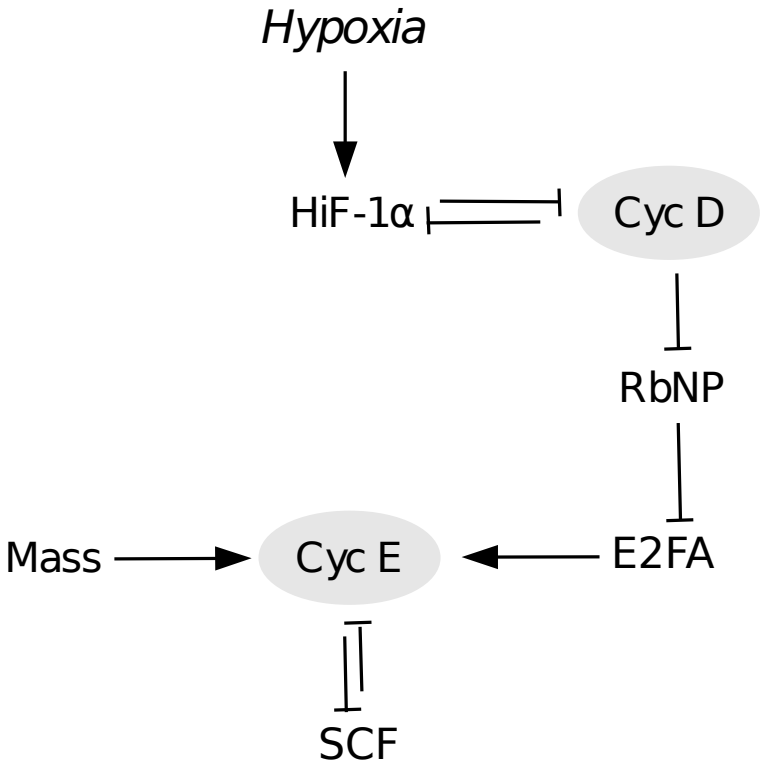

Figure 2:

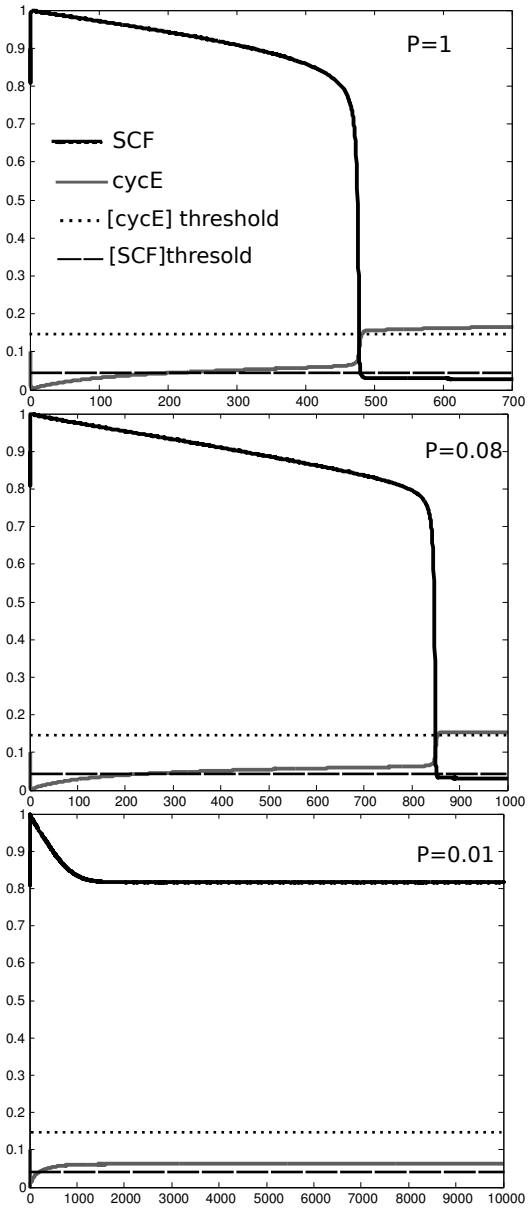

Figure 3:

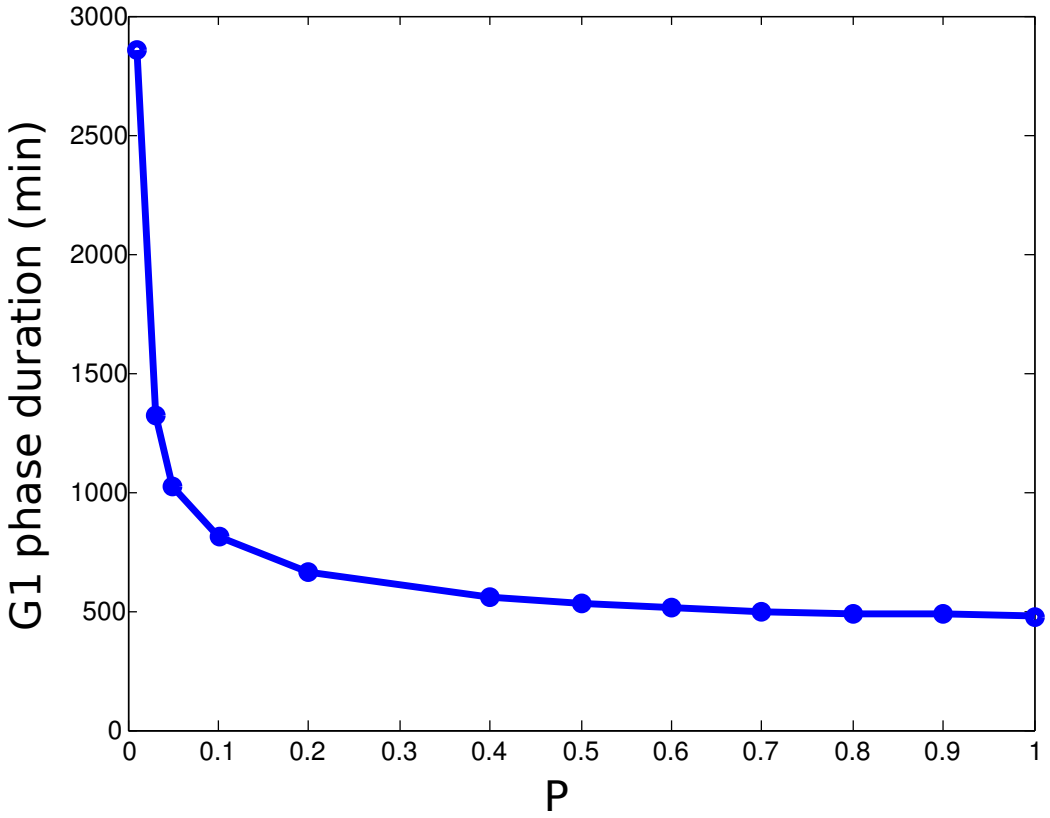
G1 phase duration (min)
3000
2500
2000
1500
1000
500
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
P

Figure 4:

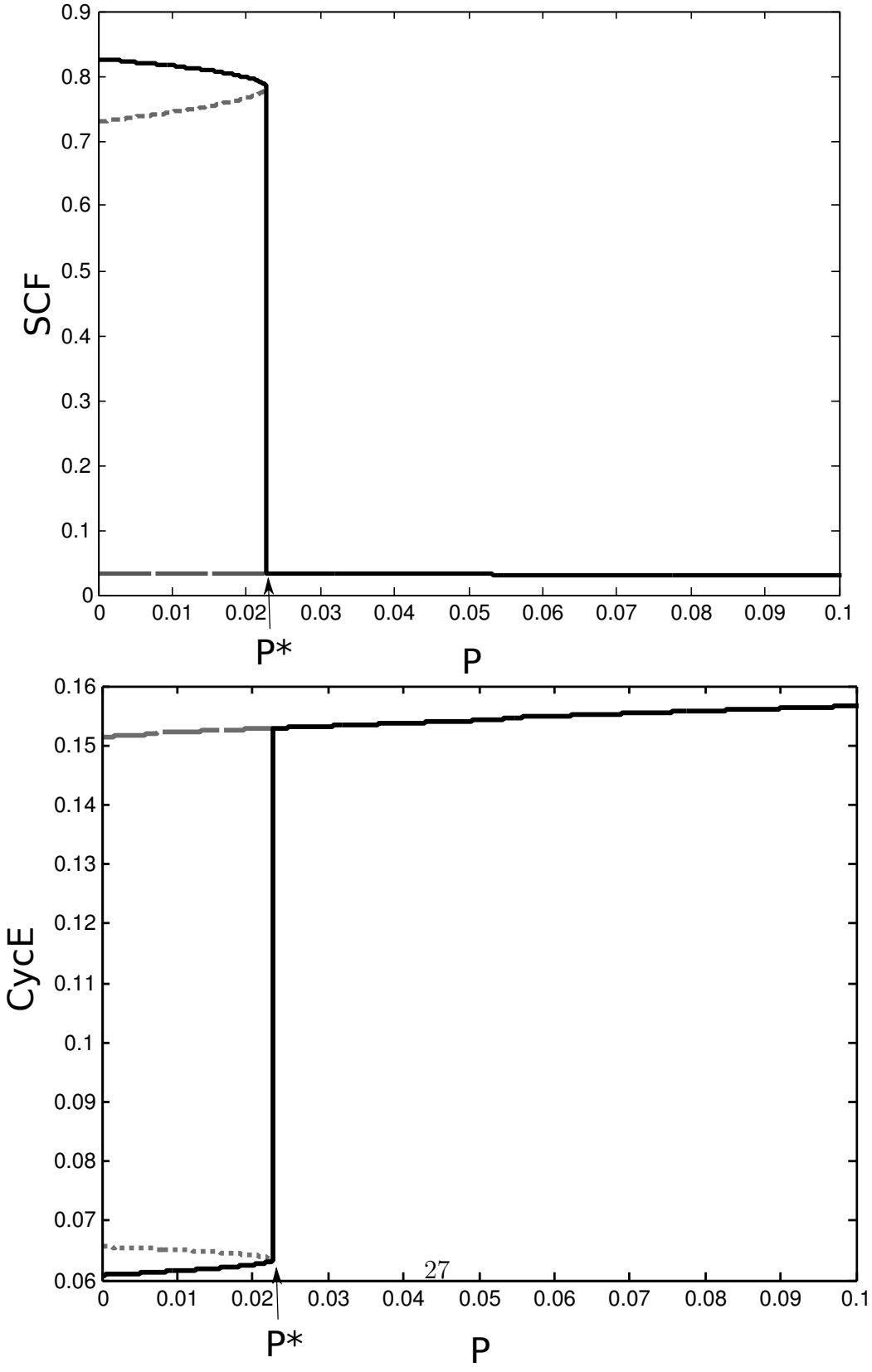

Figure 5:

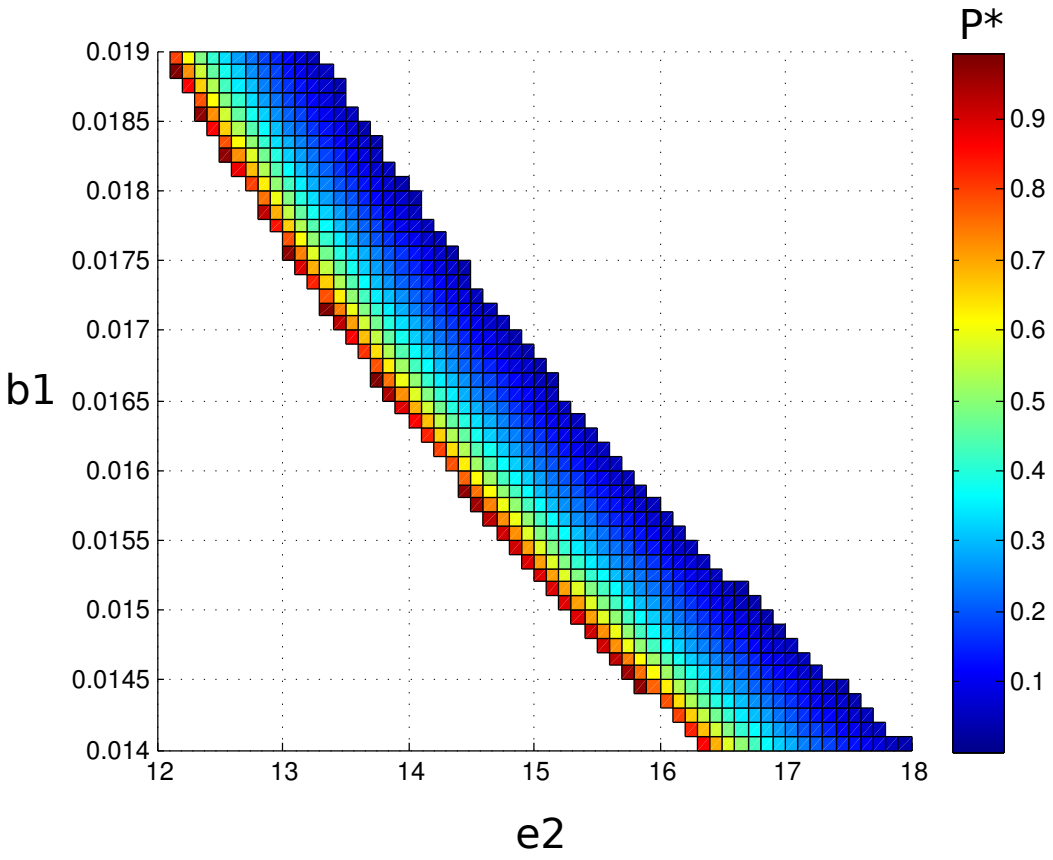
P*
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
b1
0.019
0.0185
0.018
0.0175
0.017
0.0165
0.016
0.0155
0.015
0.0145
0.014
12
13
14
15
16
17
18
e2

Figure 6:

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Appendix A. Determination of the parameters

The parameter we did not adjust were taken from [1] and [36] models, or calculated from the molecular biology literature. This first part presents the biological data used to build the model.

Appendix A.1. HiF-1α and oxygen pressure

In [27] the relationship between $[H]$ (HiF-1 concentration) and P was experimentally determined. The authors have shown that the level of HiF-1α varies with oxygen pressure in an exponential form, when the oxygen pressure is below 6%. The experimental curve they present was fitted in order to build the dimensionless expression describing the relationship between $[H]$ and P. The normoxia (P=1) is reached for an oxygen pressure P=6%.

Appendix A.2. Hif-1α and cyclin D dynamic

In [48], the level of cyclin D is quantified during the cycle: the protein is multiplied by a factor taken between 2 and 5 during the G1 phase. We adjust the maximum level of cyclin D to reach a concentration five times higher than in the beginning of the G1 phase. We also used the dynamic of the cyclin D evolution during the G1 phase modeled by [36], with a rapid increase of cyclin D concentration (maximum level reached in less than one hour). In [50] experiments were realized to quantify the inverse correlation between HiF-1α and cyclin D. Their paper shows that after 20 hours of hypoxia (oxygen pressure P=0.21%) in a cell constitutively expressing the cyclin D, its concentration reaches an equilibrium, and is divided by about 2 with respect to normoxia. Considering that our model describes cells that have endured several hours of hypoxia, and that the constitutive expression is

equivalent to the induction at the beginning of the G1 phase, the parameter a_3 was adjusted to obtain a maximal value of $[cycD]$ divided by 2 when $P = 0.21\%$.

Appendix A.3. Phosphorylation of Rb

We used the model developed by [36] to adjust the parameters controlling the quantity of phosphorylated Rb: they obtained a phosphorylation of 50% of total Rb after 4 hours of cycle.

Appendix B. Fixed points

X_∞ denotes the steady state concentration of the variable X. By equaling to zero Eqs.(1)-(6), it is possible to calculate the equilibrium values of the variables used in the model. The calculation of $cycD_\infty$, Rb_∞, $E2F_\infty$, m_∞ is straightforward:

$$\begin{aligned} cycD_\infty &= \frac{a_1 + a_3 H_0 e^{\beta_1(1-P)}}{a_3} \\ Rb_\infty &= \frac{d_2}{d_2 + d_1 cycD_\infty} \\ m_\infty &= m_0 \\ E2F_\infty &= [E2F]_{total} \end{aligned} \tag{B.1}$$

By equaling to zero Eqs.(2) and (3), we obtain an equation for $cycE_\infty$:

$$cycE_\infty = \frac{b_1 m_0 [E2F]_{tot}}{b_2 + b_3 SCF_\infty}$$

and a third degree equation for SCF_∞:

$$\begin{aligned} 0 &= -b_3 e_1 0 cyc E_\infty^3 + cyc E_\infty^2 (b_3 e_1 - e_1 J_2 b_3 - e_1 b_2 + e_2 b_1 m_0 [E2F]_{tot}) \\ &+ cyc_\infty (e_1 J_2 b_3 - e_1 J_2 b_2 - e_2 J_1 b_1 m_0 [E2F]_{tot} - e_2 b_1 m_0 [E2F]_{tot}) \\ &+ e_1 J_2 b_2 \end{aligned} \tag{B.2}$$

The equation B.2 is solved numerically, for different values of P and of the parameters α, b_1, e_2. The resolution gives us the number and the values of the fixed points; we also numerically determined the stability of these fixed points.

Table legends

Table 1: Parameters used in the model. The parameters we could not estimate are initially determined in order to reach a G1 phase duration of about 400 min for proliferative cells in normoxic conditions.

Table 2: Initial values taken for the simulations.

Figure legends

Figure 1: The biological switch between cyclin E and its inhibitor is necessary to pass through the G1/S restriction point. Schematic diagram showing the evolution of the concentration of SCF and cyc-CDK during the cell cycle. The vertical dotted line represents the restriction point: when the cell achieves the switch, it finishes the cycle. From [1].

Figure 2: Model of the G1/S transition in hypoxic conditions: schematic representation of the molecular network considered in our model. We modeled the action of cyclin D, which phosphorylates Rb and liberates E2F. This transcription factor activates the synthesis of cyclin E, which is degraded by SCF complex. HiF-1α negatively regulates cyclin D synthesis. RbNP=Non-phosphorylated Rb.

Figure 3: Evolution of $[SCF]$ (solid black line) and $[cycE]$ (solid grey line). The simulations were made for adimensionalized values of P, with P=1 (top), 0.08 (center), 0.01 (bottom). When the molecular switch occurs, the cell passes through the G1 phase. For P=0.01, the system does not generate a switch any more. The cell enters into a quiescent state.

Figure 4: Influence of hypoxia on the G1 phase duration. Value of the G1 phase duration (in min) given by the model as a function of the parameter P (adimensionalized).

Figure 5: Influence of P (adimensionalized) on the stable fixed points of the system. $[SCF]_\infty$ (top) and $[cycE]_\infty$ (bottom) are plotted as functions of P^*. The dark line represent the stationary states reach by the system with the parameters we used. The point where we pass from three to one stable stationary state corresponds to P^* (limit of the quiescent state).

Figure 6: Influence of b_1 (synthesis rate of $cycE$) and e_2 (coupling between $cycE$ and SCF) on the value of the bifurcation point P^*. The value of P^* (color scale) is plotted as a function of b_1 and e_2. The white zones correspond to non physiological values (the cell never or always finishes its cycle).